

· APPLICANTS: DiTullio et al.
U.S.S.N.:

In the Specification:

Please replace paragraph on lines 5-8 on page one with the following paragraph.

-- This application is a Continuation of U.S. Patent Application Serial No. 09/247,246, filed February 9, 1999. This application also claims priority from provisional patent application serial number 60/073,386, filed on February 9, 1998, the contents of which are hereby incorporated by reference.--

Please replace the paragraph bridging pages 3-4 with the following paragraph.

--Preferably, the non-human animal is a sheep, goat, pig, cow, chicken, rabbit, rat, mouse, or guinea pig. More preferably, the animal is prepubetal prepubertal, e.g., at an age at which the testicle has not yet begun to produce sperm. For example, the preferred age of a pig is at least 30 days but not greater than 100 days. At this age, the number of target cells, i.e., spermatogonia, is relatively low. An advantage of this approach is that destruction of spermatogenic cells prior to administration of DNA is not required.--

Please replace the paragraph on page 4, lines 3-20, with the following paragraph.

--Also within the invention is a method of making a non-human transgenic animal ~~comprising~~ by infusing DNA *in situ* into a testicle of a prepubetal prepubertal non-human animal, harvesting sperm cells from the animal, contacting an ovum with said the cells under conditions suitable for fertilization, and producing a non-human transgenic animal. By "transgenic non-human animal" is meant an animal that has gained (or lost) a DNA sequence from the introduction of an exogenous DNA sequence, i.e., transgene, into its own cells, or into an ancestor's germ line. Such animals are produced by natural breeding, artificial insemination, or

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in vitro sperm injection into ova. By the term "transgene" is meant any exogenous DNA sequence which is introduced into both the somatic and germ cells or only some of the somatic cells of a mammal. The transgene is integrated into a chromosome. If the transgene is integrated into a chromosome, it may or may not be located at the same site as its corresponding endogenous gene sequence. --

Please replace the paragraph bridging pages 6-7 with the following paragraph.

--For example, the method is carried out as follows. A male animal is anesthetized with a suitable anesthetic to achieve for example, the method is carried out as follows. A male animal is anesthetized with a suitable anesthetic to achieve a 30-60 minute time period to complete the procedure (suitable anesthetics are known in the art, e.g., goats, valium/ketamine; rabbits, acepromazine/rompun; pigs, telazol/rompun. The optimum age for the male is ~~prepubetal~~ prepubertal to reduce the number of target cells (e.g., stem/progenitor cells) to be transfected. However, the procedure is also useful for DNA delivery to spermatogonia of adult or mature animals. Once the animal has been anesthetized, DNA is introduced into the testes using a small gauge needle. The volume of DNA and concentration varies depending on the size of the testes and the efficiency of transfection. The transfection of the DNA is achieved using a variety of standard techniques and DNA formulations. For example, the DNA is infused in complex with lipids, other compounds known to enhance transfection, or uncomplexed (i.e., naked). If DNA is infused in sterile distilled water, it may be electroporated into the cells *in vivo* by passing a current across the testes using an electroejaculator, heart defibrillator, or any suitable source of electrical current. After the animal has recovered, semen is collected and analyzed for the presence of the transgene. Once the transgene has been detected, the male will be bred to

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females to produce transgenic offspring. The time between infusion of the DNA and breeding varies depending on such factors as the age of the animal at infusion, age of sexual maturity, and the time required for differentiation from stem/progenitor cell to sperm cell.--